



A Comparative Analysis-to Investigate the Antioxidant Profiles of Various Peels Using Different Extraction Solvents

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Abstract

There's been a growing interest in substituting synthetic food antioxidants with natural alternatives, leading to research on vegetable sources and screening of raw materials to discover new antioxidants. The aim of this study was to explore how two commonly used solvents impacted the yields of phenolics, flavonoids, and the antioxidant properties of extracts from eggplant, beetroot, and potato peel. Among the three extracts, eggplant showed the highest percentage of phenolic content (64.3 mg/g), total flavonoid content (0.8 mg/g), and antioxidant activity (75.6%) when extracted with ethanol compared to beetroot and potato peel extracts. Various antioxidant-related phytochemical compositions, including Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), were analyzed. Additionally, antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging. The findings indicated that ethanol, as a solvent, exhibited the most robust antioxidant profile among the three peels. Given that different antioxidant compounds operate through diverse mechanisms, based on these results, extracts from eggplant, beetroot, and potato peel could serve as natural antioxidants due to their significant antioxidant activity. Consequently, they might be utilized as preservative ingredients in the food and/or pharmaceutical industries.

Introduction

Antioxidants are naturally present in various foods and are crucial for maintaining our health. They encompass nutrients like Vitamin C from fruits and vegetables and Vitamin E from seeds and nuts. In the food industry, both natural and synthetic antioxidants are utilized as additives to extend the shelf life and preserve the appearance of many food items, particularly those containing vegetable or animal fats prone to oxidation from oxygen, heat, moisture, or enzymes.

The pace of oxidation depends on factors such as the origin of the oil or fat and storage conditions. Most vegetable oils contain inherent antioxidants like vitamin E. While synthetic antioxidants are predominantly employed for this purpose, it's worth noting that fruit and vegetable processing in India yields significant waste. Research has shown that these by-products, such as peels and pomace, are rich sources of antioxidant polyphenols, along with sugars, minerals, organic acids, dietary fibers, and phenolics, which exhibit various beneficial properties, including antioxidative, antimutagenic, cardio preventive, antibacterial, and antiviral effects.

Antioxidants found in fruits and vegetables, including ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins, are believed to play a pivotal role in disease prevention. Eggplant, a staple of the Mediterranean diet, ranks among the top ten vegetables for its capacity to scavenge oxygen radicals, thanks to its phenolic constituents. It contains chlorogenic acid and Nasunin, both potent antioxidants that combat free radicals implicated in aging, inflammation, cardiovascular diseases, and cancer.

Similarly, beetroot, derived from the beet plant, is renowned for its taproot, which is consumed as a vegetable. Besides its culinary use, beets serve as a natural food coloring and have medicinal properties. The potato, a starchy tuberous crop, yields waste primarily from peeling, trimming, and processing operations, leading to pollution issues. Potato peels have been identified as containing phenolic acids, although the evidence supporting their free radical-scavenging activity is not yet conclusive.

MATERIALS AND METHODS

Materials

Raw materials: Eggplant, Beetroot and Potato peels were obtained from the local market, Allahabad, India. The comparative study on antioxidant profile of different peels by using different extraction solvent, was carried out in Department of Food Process Engineering, Vaugh School of Agricultural Engineering and Technology, SHIATS.

Experimental Procedure:

Extraction of antioxidant from peels:

By using ethanol as a solvent

The dried powders of peels were extracted by cold percolation method using ethanol as a solvent, (Parekh and Chanda, 2007)

By using 80% aqueous methanol as a solvent

The dried powders of peels were extracted by using 80% aqueous methanol as a solvent, (Mazza and Gao, 1998)

Determination of Extraction yield:

The residues obtained after filtration were weighed to obtain the extraction yield. Extraction yield (%)= (weight of the residue)/(total weight of the peel powder)×100

Determination of Total Phenolic:

The total phenol content was determined according to Folin- Ciocalteu's reagent method (Mc Donald *et al.*, 2001). 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml of 20% sodium carbonate solution was added and further incubated for 30 min. at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve: $y = 1.170 x - 0.012$; $R^2 = 0.987$, where y is the absorbance and x is concentration as gallic acid equivalents (mg/g). Total phenol values are expressed in terms of gallic acid equivalent

Determination of Total Flavonoids:

The flavonoid content was determined according to aluminium chloride colorimetric method (Chang *et al.*, 2002). The reaction mixture consisting in a final volume of 3 ml, 1.0 ml of sample(1 mg/ml) 1.0ml methanol and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Quercetin was used as positive control (Kaneria *et al.*, 2009). Flavonoid content is expressed in terms of Quercetin equivalent. Total flavonoid content were calculated as quercetin (mg/g) using the following equation based on the calibration curve:

$y = 0.145 x + 0.055$; $R^2 = 0.993$, where x was the absorbance and y was the quercetin equivalent (mg/g).

DPPH radical-scavenging activity:

The DPPH assay was utilised with some modifications. The stock reagent solution (1×10^{-3} mol L⁻¹) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20 C until use. The working solution (6×10^{-5} mol L⁻¹) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extract and synthetic antioxidant (TBHQ, BHA and BHT in ethanol) solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

DPPH radical-scavenging activity (%)= [(Acontrol - Asample)/Acontrol] × 100 where A is the absorbance at 515 nm.

Table 1 Statistical analysis for comparative analysis of antioxidant profile using ethanol assolvent

| Peels | Total Phenolic Content (mg/g) | Total Flavonoid Content (mg/g) | Antioxidant Activity (%) |
|---------------|-------------------------------|--------------------------------|--------------------------|
| Eggplant Peel | 64.3 | 0.8 | 75.6 |
| Beetroot Peel | 13.6 | 0.7 | 66.3 |
| Potato Peel | 14.5 | 0.5 | 86.3 |
| F-Test | S | S | S |
| S.Ed (±) | 0.308 | 0.006 | 0.760 |
| C.D (P=0.05) | 0.616 | 0.013 | 1.531 |

Table 2 Statistical analysis for comparative analysis of antioxidant profile using 80%aqueous methanol as solvent

| Peels | Total Phenolic Content (mg/g) | Total Flavonoid Content (mg/g) | Antioxidant Activity (%) |
|---------------|-------------------------------|--------------------------------|--------------------------|
| Eggplant Peel | 44.7 | 0.3 | 65.9 |
| Beetroot Peel | 11.9 | 0.2 | 50.4 |
| Potato Peel | 15.3 | 0.7 | 70.2 |
| F-Test | S | S | S |
| S.Ed (±) | 0.239 | 0.004 | 0.621 |
| C.D (P=0.05) | 0.479 | 0.008 | 1.243 |

Statistical Analysis

All experiment was determined 3 times and the results were reported as mean. The data recorded during the course of investigation were statistically analyzed by the 'Analysis of Variance- One Way Classification'.

RESULTS AND DISCUSSION

Proximate composition of materials

Moisture, protein, fat, ash, were determined according to standard methods (AOAC, 2005) given in Ranganna, 1986 respectively.

Table 3 Statistical analysis for comparison in nutritional value of different peels.

| Peels | Moisture Content (%) | Fat (%) | Ash (%) | Protein (%) |
|---------------|----------------------|---------|---------|-------------|
| Eggplant Peel | 8.49 | 1.15 | 4.42 | 0.52 |
| Beetroot Peel | 7.92 | 0.25 | 4.11 | 0.25 |
| Potato Peel | 6.84 | 0.15 | 4.05 | 0.05 |
| F-Test | S | S | S | S |
| S.Ed (±) | 0.077 | 0.005 | 0.041 | 0.0027 |
| C.D (P=0.05) | 0.161 | 0.010 | 0.083 | 0.0054 |

Total Phenolic Content & Total Flavonoid Content when extracted with ethanol/80%aqueous methanol as solvent:

TPC was found maximum in eggplant peel (64.3 mg/g), minimum in beetroot peel (13.6 mg/g) whereas potato peel showed the TPC of (14.5 mg/g). When extracted with ethanol as a solvent. TPC was found maximum in eggplant peel (44.7 mg/g), minimum in beetroot peel (11.9 mg/g) whereas potato peel showed the TPC of (15.3 mg/g). When extracted with 80% aqueous methanol as a solvent. (Somawathi and Rizliyal, 2014) TFC found in eggplant peel (0.8 mg/g), in beetroot peel (0.7 mg/g) whereas potato peel showed the TFC of (0.5 mg/g) when extracted with ethanol as solvent. TFC found in eggplant peel (0.3 mg/g), minimum in beetroot peel (0.2 mg/g) whereas potato peel showed the TFC of (0.7 mg/g). When extracted with 80% aqueous methanol as a solvent. (Mohamad and Fatma, 2010) The results were in agreement with who studied the process for extraction of antioxidants from peel, but differed slightly this may be due to the experimental and environmental conditions. The concentration of phenolics and flavonoids in the extracts, expressed as mg of GAE/g sample was dependent on the solvent and method used in the extraction. The amount of phenolic compound in the ethanolic extract was highest and total phenolics concentration in the two solvents were in the order: Ethanol > 80% aqueous methanol. Peels contain many phenolics compounds, some are in free form and some are in bound form. The major phenolics acid in the peel extract were identified as chlorogenic acid (CGA), gallic acid (GAC), protocatechuic acid (PCA), and caffeic acid (CFA).

Total antioxidant activity

Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases. DPPH is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH, reducing the number of DPPH• free radicals to the number of their available hydroxyl groups. Therefore the absorption at 515 nm is proportional to the amount of residual. The extracts that showed relatively high antioxidant activity (those with methanol and ethanol), as strong as that of BHA and BHT but weaker than that of TBHQ, contained the highest amount of total phenolic compounds.

The results of the DPPH free radical-scavenging assay suggest. That components within the extracts are capable of scavenging free radicals via electron-or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices, e.g. biological membranes. This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. DPPH. It is visually noticeable as a discolouration from purple to yellow. The scavenging activity of extracts against DPPH was concentration-dependent. Significant ($P < 0.05$) differences between extracts were observed, but the results clearly indicate that all extracts exhibited antioxidant activity. The extracts that showed relatively high antioxidant

CONCLUSION

Antioxidant activity of different peels have health promoting and disease-preventing effects. Consequently, consumption of eggplant, beetroot and potato which contains polyphenols, from eggplant, beetroot and potato may have a potential therapeutic use. This study confirms that the phenols and flavonoids are present in various parts of vegetables used, particularly in skin, suggesting the use of the entire vegetable as food. Extraction may be an attractive alternative to conventional method to improve the amount of polyphenols and the antioxidant activity extracted, in particular for nutraceutical application. Extraction of skin with ethanol was particularly effective as compared to 80% aqueous methanol.

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